Seed bank dynamics in restored grassland following the sowing of high- and low-diversity seed mixtures

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Abstract

Soil seed banks on ex-arable land are dominated by undesirable ruderal species that compete with ‘desirable’ target species during grassland restoration. At the same time, for continued regeneration, the latter often functionally depend on gap colonization from the seed bank, which serves as a buffer against local extinction. Nonetheless, few studies have so far investigated the effects of restoration practices on seed bank dynamics. Using a multi-site experiment investigating techniques for restoring lowland mesotrophic grassland, we studied the effects of seed bed preparation (shallow cultivation using harrows or discs vs deep cultivation using a plough) and of seed mixtures (species-rich grass-forb mixes vs species-poor grass-only mixes vs unseeded natural regeneration) on seven years of post-restoration seed bank dynamics. We assessed how these practices affected density and diversity of sown and unsown species in the seed bank. Seed bank dynamics were much more strongly affected by seed sowing than by cultivation. Grass sowing resulted in stronger seed bank decline of unsown grasses, and additional forb sowing in stronger decline of unsown forbs. Higher seed densities and species richness of sown forbs colonizing from neighboring plots sown with the grass-forb mix were observed under natural regeneration than in the grass-only sown treatment, reflecting grass priority effects on sown forb colonization in the latter. Sowing of diverse target species mixtures was associated with the greatest shift in seed bank composition away from extant ruderal species towards sown target species. Our results illustrate the usefulness of seed bank monitoring for assessing restoration progress.

Keywords: cultivation; ex-arable land; natural regeneration; priority effects; sowing

Implications for Practice:

- Use of seed mixes in grassland restoration can accelerate development of a functional seed bank that is more reflective of the target community, by promoting decline of unsown non-target species and accumulation of sown target species in the seed bank.
- Species-rich grass-forb mixtures appear to be more effective than species-poor grass-only mixtures, by enabling a faster build-up of sown forbs in the seed bank, along with a faster decline of unsown non-target forbs.
- Ploughing to regular depth as opposed to shallow cultivation does not appear to have any positive effects on seed bank or vegetation development.
- Complementation of vegetation monitoring during restoration by additional seed bank monitoring can help obtain a more integrated picture of restoration progress.

Introduction

Soil seed banks play an important functional role in the continuous regeneration of plant populations (Thompson 2000). In grassland, this is illustrated by the fact that re-colonization of small-scale canopy openings such as those created by sward management is often effected by the soil seed bank (Pakeman et al. 1998; Kalamees & Zobel 2002; but see also Bullock et al. 1994; Edwards & Crawley 1999). It is also known that the above-ground persistence of grassland species is positively linked to their seed bank persistence (Stöcklin & Fischer 1999), underlining the fact that buried seed populations act as a buffer against localised extinction during periods characterized by unfavourable conditions.

When semi-natural grassland is restored on former arable land, the soil seed bank tends to be dominated by ruderals, whereas typical species of the grassland target are usually absent (Walker et al. 2004). This can have implications for success of restoration of target species, which, amongst others, will be affected by the surface-layer densities of the seeds both of target species and of potentially undesirable non-target species (Walker et al. 2004). Accordingly, some authors have suggested soil inversion as a means of reducing numbers of seeds of unwanted species, by burying them deeper in the soil profile and thereby preventing them from interfering with the establishment of target species (Glen et al. 2007; Czerwiński et al. 2015; Glen et al. 2017).

At newly-restored sites, the initial absence of sown target species from the seed bank increases the vulnerability to environment and management factors with the potential to cause of newly-established populations due to demographic events and to buffer against trigger such events.

100 A rapid build-up of target species' seed populations in the soil seed bank, coupled
101 with a steady depletion of the seeds of non-target species, is thus desirable and might
102 positively affect restoration progress. However, little is known about how grassland
103 restoration techniques on ex-arable land, such as seed sowing or type of soil
104 cultivation, influence seed bank dynamics both in terms of unsown and sown species.
105 Few studies have investigated seed bank dynamics in restored grassland (Rayburn et
106 al. 2016), especially on ex-arable land (but see McDonald et al. 1996; Schmiede et al.
107 2009; Fagan et al. 2010; Török et al. 2012; Karlík & Poschlod 2014). Of the few
108 studies that have, none assessed the effects of cultivation, and only Fagan et al. (2010)
109 assessed the potential effects on seed banks of seeding with different seed mixtures.
110 However, Fagan et al. (2010) made no distinction between sown and unsown species,
111 as many of the sites included had been seeded using green hay or other plant materials
112 collected from donor sites, and species composition of these materials is usually only
113 known approximately, and often highly variable.
114 The work presented here, carried out as part of a study into the use of soil
115 cultivation and the sowing of different kinds of seed mixtures for re-creating species-
116 rich mesotrophic grassland on ex-arable land (Pywell et al. 2002), aims to help closing
117 this gap in our knowledge of seed bank dynamics during restoration. Based on
118 repeated sampling of the soil seed bank across a range of treatments in Pywell et al.'s
119 (2002) experiment, we set out to investigate the following questions:
120 (1) To what extent does the sowing of seed mixtures of different diversity affect
121 dynamics of unsown and sown target species in the soil, and how do these approaches
122 compare with natural regeneration?
123 (2) To what extent does seed bed preparation (deep cultivation versus shallow
124 cultivation) affect seed bank dynamics?

(3) To what extent are compositional trends in the seed bank affected by experimental treatments, and how do these trends relate to compositional trends in the aboveground vegetation?

Methods

Field sites and experimental design

Three experimental sites located in southern England were included in this study (Table 1). At these sites, experimental restoration was carried out from September 1994 to determine the relative success of various cultivation methods and seed sowing options when establishing species-rich grassland on ex-arable land (Pywell et al. 2002). Sites were selected in natural areas that had been included in the UK's Environmentally Sensitive Areas (ESA) scheme, an agri-environment scheme that ran from 1986 to 2005 and whose aims included both the protection of remaining diverse grasslands as well as at the creation of additional grassland habitat of high biodiversity value (Coates 1997). For the experiment, a specific lowland grassland target community conforming to the British National Vegetation Classification (Rodwell 1992) was identified for each site depending on location, soil, hydrology and proposed management. For all three sites included here, targets represented specific sub-communities of *Cynosurus cristatus – Centaurea nigra* grassland (NVC category MG5; Table 1). MG5 grassland corresponds to the continental European *Centaureo-Cynosuretum cristati* Br.-Bl. & Tx grassland association (Rodwell 1992), and the most frequent constant species in this community are the grasses *Agrostis capillaris*, *Cynosurus cristatus* and *Festuca rubra*, and the forbs *Lotus corniculatus*, *Plantago lanceolata* and *Trifolium repens* (Rodwell 1992). *Centaurea nigra*, in spite of being one of the species the community is named after, occurs somewhat less

150 frequently (Rodwell 1992). For two of our sites, the Norfolk Broads site and the
151 Upper Thames Tributaries site, we did identify the MG5a sub-community as suitable
152 target for restoration, whose preferential species include *Lathyrus pratensis* and
153 *Leucanthemum vulgare* (Rodwell 1992). For the third site, the Suffolk River valleys
154 site, we did identify the MG5c sub-community as suitable target, which amongst
155 others is characterized by preferential species including *Danthonia decumbens*,
156 *Potentilla erecta*, and *Succisa pratensis* (Rodwell 1992).
157 Specifically, Pywell et al. (2002) compared pre-sowing cultivation techniques,
158 namely no cultivation vs. shallow cultivation vs. deep cultivation, and the use of
159 different seed mixtures, namely natural regeneration without sowing vs. species-poor
160 grass-only mixture (= 'ESA mixture') vs. species-rich mixture including grasses and
161 forbs that are characteristic of the respective NVC target community (= 'NVC
162 mixture'). NVC mixtures differed between the Norfolk Broads and Upper Thames
163 Tributaries sites, which had an MG5c restoration target, and the Suffolk River Valleys
164 site, which had MG5a grassland as restoration target. At the former two sites, NVC
165 mixtures included 11 grasses and 28 forbs, and at the latter eight grasses and 17 forbs,
166 reflecting the slightly smaller pool of characteristic species in MG5c grassland
167 (Rodwell 1992; for full details see Table S1, Supporting Information). The same ESA
168 mixture of six grasses was used at all three sites, with one additional species,
169 *Alopecurus pratensis*, included at the Norfolk Broads site (Table S1). The ESA
170 mixture was based on Department of Environment, Food and Rural Affairs (Defra)
171 guidelines for establishing moderately diverse grass cover on arable land. NVC
172 mixtures were made up of grasses and a sizeable number of forb species based on the
173 composition of the chosen target community. Depending on soil type, shallow
174 cultivation was achieved with harrows or discs, with a maximum cultivation depth of

10 cm and no soil inversion. Deep cultivation consisted of ploughing to a depth of 30-40 cm, which inverted the uppermost soil layer. This is similar to conventional agricultural ploughing and is not to be confused with the more recently developed restoration practice of 'deep-ploughing' which typically involves cultivation to a depth of about 80 cm (Glen et al. 2017). The experiment was set up in four replicate blocks per site, and treatment plots were 6 m × 4 m with a 1 m guard row between the plots (Pywell et al. 2002). Here, we focus on five of the seven treatments included in Pywell et al.'s (2002) study:

(1) natural regeneration from cereal stubble (i.e. a control with no cultivation or sowing of species);

(2) shallow cultivation + species-poor ESA seed mixture;

(3) shallow-cultivation + species-rich NVC seed mixture;

(4) deep cultivation + species-poor ESA seed mixture;

(5) deep cultivation + species-rich NVC seed mixture.

Vegetation monitoring

Vegetation sampling was carried out annually from 1995 to 1998. In early July of each year, three 40 cm × 40 cm quadrats, subdivided into 16 cells of 10 cm × 10 cm, were placed at random within each plot, avoiding a 1 m buffer strip around the edge. The presence of vascular plant species was recorded for each cell. *Festuca rubra* and *Festuca ovina* were treated as an aggregate due to difficulties in distinguishing between these species. Nomenclature follows Stace (2010).

Seed bank sampling and monitoring

In September 1994 after cultivation, and again in October 1998 and October 2001, soil was sampled to a depth of 20 cm in all experimental plots. This was done for the seed bank analyses presented here as well as for nutrient analyses (for these see Pywell et al. 2002), using a soil auger of 6 cm diameter, to extract ten randomly placed soil cores per treatment plot. After removal of the litter layer, the soil cores were divided into segments of 0-5 and 5-20 cm depth. The 10 segments for a given experimental plot were pooled for each depth, and pooled plot samples thoroughly mixed.

From each plot sample, a volume of 500 cm^3 was sub-sampled for seed bank analyses, from which large vegetative fragments, roots and stones were removed. Sub-samples were then transferred into 20 cm × 16 cm plastic trays where they were spread evenly in a layer of ca. 1.5 cm thickness over a 3 cm layer of sharp sand. In 1994, seed bank analysis was only carried out for the 0-5 cm surface layer, whereas in 1998 and 2001, it was also carried out for the 5-20 cm sub-surface layer. However, here, we focus entirely on the 0-5 cm surface layer. About 5 cm is usually the maximum depth from which the seedlings of grassland species can emerge (Williams 1983; Traba et al. 2004), and the 0-5 cm top layer of soil can thus be considered to represent the functional seed bank of undisturbed grassland.

After processing, seed trays were transferred onto a bench in a heated glasshouse, along with four randomly placed trays per year only containing a layer of sharp sand, to check for potential contamination by airborne seed or by seed emerging from the sand layer. However, throughout the study, no seedling emergence was observed from these control trays.

All trays were watered initially, and subsequently kept moist using automated irrigation. Seedlings were periodically identified and removed for a 12-month period,

during which the soil was thoroughly stirred every two months. If a seedling was not identifiable, it was transplanted into a flower pot and grown until identification was possible.

Data analysis

Seed bank density and species richness

Treatment effects on seed densities and species richness in the soil seed bank were analysed with SAS 9.3 (SAS Institute, Cary, NC, US). Separate analyses were carried out for sown species, with sub-groupings of sown forbs and sown grasses, and for unsown species, with sub-groupings of unsown forbs and unsown grasses. The 'unsown species' grouping also contained woody species and rushes (*Juncus* spp.). However, seeds of these two sub-groupings tended to occur quite unevenly across sites, and in the case of woody species also very sporadically, and thus, no separate analyses are presented.

Prior to analyses, seedling counts were summed across member taxa of each species group. Analyses for unsown and sown species groups differed in one important respect. The former were based on data from all three samplings, whereas the latter were based on data from 1998 and 2001 only, as sown species were entirely absent from the seed bank in 1994. In terms of interpreting analysis results, this means that for unsown groups, for which a pre-treatment baseline was included in analyses, treatment effects should primarily manifest themselves as interactions between treatment and year. In contrast, for sown species groups, treatment effects may likely manifest themselves also as main effects. The earliest data included in these analyses was from four years after sowing, by which time some treatment effects may have already occurred.

Because the five restoration treatments included were not fully factorial, we carried out two kinds of analysis, henceforth referred to as analysis A and analysis B. Analysis A focused on effects of seed sowing (natural regeneration vs. ESA seed mixture vs. NVC seed mixture), including treatments (1) to (3). The shallow cultivation prior to sowing in treatments (2) and (3) can be considered as the minimum seed bed treatment, and is thus most comparable to natural regeneration (Pywell et al. 2002). Analysis B focused on effects of seed mixture (ESA vs. NVC) and cultivation depth (deep vs. shallow), including treatments (2) to (5).

Together with experimental factors, which were specified as fixed factors in the respective analyses, site and year were also specified as fixed factors, along the various interaction terms between these main factors. In both types of analysis, year was specified as repeated measures factor, and blocks nested within sites were included as random effects (Schabenberger & Pierce 2002).

Analyses were carried out using GLMMs and Poisson errors as provided by SAS Proc GLIMMIX. If GLMMs failed to converge, alternative analyses were carried out using computationally less demanding LMMs as provided by SAS Proc MIXED. In this case, while models were specified in the same way, data was Box-Cox-transformed prior to analysis to meet distributional requirements. In case of a significant main effect of seed sowing for analyses A, pair-wise comparisons between the three seed sowing treatments were carried out using the two-sided Tukey HSD test.

Compositional trends

To analyse compositional trends in seed bank and vegetation, we performed an ordination analysis using Non-metric Multidimensional Scaling (NMDS) as provided by PC-ORD, Version 6.08 (McCune & Mefford 2011). Prior to analysis, for each

274 species, quadrat cell counts from the three replicate quadrats in a given plot and year
275 were summed. Then, to make seed bank and vegetation data comparable, all data was
276 standardized to the sample total (Wagner et al. 2006).
277 As recommended by McCune and Grace (2002), NMDS was performed using
278 the Sørensen distance measure. Optimal dimensionality for the final model was
279 assessed with the 'slow and thorough' option of PC-ORD's autopilot mode (McCune
280 & Grace 2002). The final run was carried out with a predefined stability criterion of
281 0.000001 and a maximum of 500 iterations. For each of the resulting NMDS axes, we
282 determined the amount of compositional variance explained by calculating R^2
283 correlation coefficients between distance in NMDS ordination space and distance in
284 raw data space (McCune & Grace 2002). Species scores were calculated on the basis
285 of weighted averages of site scores.
286 To help evaluate compositional trends in the vegetation in terms of restoration
287 progress, we calculated percentage fit of the vegetation in each treatment and site for
288 each year with the respective target community of the NVC classification (see Table
289 1), based on the species lists provided by Rodwell (1992) and using the bespoke
290 Tablefit software, Version 2.0 (Hill 2015). This was done based on quadrat
291 frequencies (Hill 2015; see also Hill 1989), based on the twelve quadrats (= three
292 replicate quadrats × four replicate plots) per treatment in a given year at a given site.
293 Results of these goodness-of-fit calculations are presented in Fig. S1, Supporting
294 Information.
295
296 **Results**
297 A total of 5750 seedlings emerged from all soil samples across all three sample years
298 (1994: 1755 seedlings; 1998: 1751 seedlings; 2001: 2244 seedlings). Seedlings of 103

299 taxa were identified, including 10 sown grasses and 14 sown forb species. We were
300 unable to identify 368 seedlings (= 6.4% of the total), including 353 forb and 15 grass
301 seedlings. Of these 368 seedlings, 341 were from samples collected in 1994 prior to
302 sowing. As sampling in 1994 was carried out before seed mixes were sown,
303 unidentified seedlings from that year were included in the respective 'unsown'
304 categories, as excluding these seedlings from analysis in spite of knowing that they
305 must be from unsown species would have carried a risk of biasing the results. In
306 contrast, the 27 unidentified seedlings from later years were not included in analyses
307 in the default analyses presented here. Additional analyses in which these 27 seedlings
308 were alternatively included in the respective unsown categories (results not shown)
309 indicated that this decision did not affect analytical results.
310
311 ***Seed bank density and species richness***
312 Site-dependent effects, particularly those not acting in interaction with treatment
313 factors, are less relevant in terms of allowing general conclusions for restoration.
314 Therefore, here, we focus on the main and interaction effects, particularly with time,
315 of the experimental treatments. Full statistical results are presented in Tables S2 to S5,
316 Supporting Information.
317
318 *Effects of seed addition and mixture type: unsown species*
319 Analyses A and B indicated highly significant year effects on seed bank densities of
320 all unsown species groupings (Tables S1-S2), reflecting a decline in density with time
321 (Fig. 1). This decline was more pronounced for forbs than grasses (Fig. 1). Analyses
322 A indicated highly significant interactions between seed addition and year for unsown
323 species in general (GLMM; $F_{2,72} = 6.58$; $P < 0.001$) and for unsown grasses (LMM;

$F_{2,72}$ = 3.53; P = 0.011). In both groupings, the decline was stronger with sown seed mixtures than with natural regeneration (Fig. 1).

For unsown forbs, while analysis A yielded a non-significant interaction between seed addition and year (GLMM; $F_{2,72}$ = 2.16; P = 0.082), analysis B yielded a significant seed mix main effect (GLMM; $F_{1,99}$ = 6.04; P = 0.016), indicating slightly lower densities on plots sown with the NVC mix than on plots sown with the ESA mix (Fig. 1).

As indicated by analyses A, species richness per sample was significantly affected by seed addition for unsown species in general (GLMM; $F_{2,72}$ = 4.66; P = 0.012), and for unsown forbs (GLMM; $F_{2,72}$ = 3.24; P = 0.045), and highly significantly so for unsown grasses (LMM; $F_{2,72}$ = 11.61; P < 0.001). For the latter group, there was also a significant interaction of seed addition with year (LMM; $F_{2,72}$ = 2.81; P = 0.031). As indicated by two-sided pairwise Tukey tests, species richness of unsown species in general was lower in soil samples from NVC plots than in samples from natural regeneration plots (d.f. = 72; t = 2.93; P = 0.012; see Fig. 2). A similar trend of samples from NVC plots being less species-rich than samples from ESA plots was only marginally significant (d.f. = 72; t = 2.39; P = 0.051). Species richness of unsown forbs was lower in samples from NVC plots than in those from ESA plots (d.f. = 72; t = 2.49; P = 0.040; see Fig. 2). Species richness of unsown grasses was lower in samples from sown plots in general than in samples from natural regeneration plots (ESA mix vs. natural regeneration: d.f. = 72; t = 3.58; P = 0.002; NVC mix vs. natural regeneration: d.f. = 72; t = 4.58; P < 0.001; see Fig. 2). Accordingly, in analyses B, sowing of ESA vs. NVC mix affected species richness of unsown forbs (GLMM; $F_{1,99}$ = 4.74; P = 0.032) and of unsown species in general (GLMM; $F_{1,99}$ = 6.07; P = 0.017), but not of unsown grasses (LMM; $F_{1,99}$ = 1.95;

P = 0.166). In the case of unsown species in general, the effect of seed mix varied with year and site, as indicated by a highly significant three-way interaction (GLMM; $F_{4,99} = 4.07$; P = 0.004).

Effects of seed addition and mixture type: sown species

Seed bank densities of sown species groupings were strongly affected by seed addition, as indicated by highly significant main effects in analyses A (all P < 0.001; all sown species: GLMM; $F_{2,45} = 20.94$; forbs: LMM; $F_{2,45} = 25.87$; grasses: LMM; $F_{2,45} = 12.04$; see also Table S4). Seed densities of sown species in general were highest on NVC plots and lowest on natural regeneration plots, with ESA plots intermediate (Tukey tests with d.f. = 45; NVC vs natural regeneration: t = 6.45; P < 0.001; ESA vs natural regeneration: t = 4.21; P < 0.001; NVC vs ESA: t = 2.53; P = 0.040; see Fig. 3). Sown forb seed densities were also highest on NVC plots, but were actually lower on ESA plots than on natural regeneration plots (Tukey tests with d.f. = 45; NVC vs natural regeneration: t = 4.28; P < 0.001; ESA vs natural regeneration: t = -2.87; P = 0.017; NVC vs ESA: t = 7.15; P < 0.001; see Fig. 3). For sown forb seed densities, a significant interaction between seed addition and year (LMM; $F_{2,45} = 4.61$; P = 0.015) reflected continued increases on NVC plots between 1998 and 2001 as compared to continually low levels on natural regeneration plots. Seed bank densities of sown grasses were higher on seeded plots than on natural regeneration plots, irrespective of mixture used (Tukey tests with d.f. = 45; NVC vs natural regeneration: t = 3.44; P = 0.004; ESA vs natural regeneration: t = 4.75; P < 0.001; see Fig. 3). Accordingly, in analyses B, we found significant main effects of seed mix for sown species in general (GLMM; $F_{1,63} = 7.17$; P = 0.010) and for

sown forbs (GLMM; $F_{1,63} = 18.68$; $P < 0.001$), but not for sown grasses (GLMM; $F_{1,63} = 1.03$; $P = 0.314$).

Species richness per sample was highly significantly affected by seed addition in all three groupings (all sown species: GLMM; $F_{2,45} = 16.55$; $P < 0.001$; forbs: LMM; $F_{2,45} = 19.36$; $P < 0.001$; grasses: $F_{2,45} = 5.47$; $P = 0.008$). Overall richness of sown species was highest in NVC plots (Tukey tests with d.f. = 45; NVC vs natural regeneration: $t = 4.44$; $P < 0.001$; NVC vs ESA: $t = 4.89$; $P < 0.001$; see Fig. 4), but there was no difference between the ESA and natural regeneration treatments ($t = -0.57$; $P = 0.839$). Sown forb richness was highest in samples from NVC plots and lowest in those from ESA plots, with samples from natural regeneration plots characterized by intermediate richness (Tukey tests with d.f. = 45; NVC vs natural regeneration: $t = 3.43$; $P = 0.004$; NVC vs ESA: $t = 6.21$; $P < 0.001$; ESA ns natural regeneration: $t = -2.78$; $P = 0.021$; see Fig. 4). Sown grass richness was significantly higher in samples from NVC plots than from natural regeneration plots (d.f. = 45; $t = 3.43$; $P = 0.004$), with sown grass richness in samples from ESA plots being intermediate and not significantly different from that in the other two treatments. Results of analyses B were mostly in agreement with those from analyses A, with significant main effect of seed mix on overall sown species richness (GLMM; $F_{1,63} = 37.64$; $P < 0.001$) and on forb richness ($F_{1,63} = 18.73$; $P < 0.001$). However, with analysis B being based on data from both the shallow-cultivated plots and the deep-cultivated plots, unlike in analysis A, we found a significant seed mix effect for grass richness ($F_{1,63} = 8.89$; $P = 0.004$) that was indicative of higher richness in samples from NVC plots than from ESA plots (Fig. 4).

Effects of cultivation

Both for unsown and sown species groupings, there were only few main or interaction effects of cultivation (Tables S3 and S5). For seed density of unsown grasses, there was a significant cultivation main effect (LMM; $F_{1,99} = 6.23$; $P = 0.014$), reflecting slightly higher seed densities on deep-cultivated plots than on shallow-cultivated plots (Fig. 1). In addition, we found one significant three-way interaction involving cultivation for unsown species richness (Table S3) and one for sown species richness (Table S3). Other than that, we found no evidence for choice of cultivation to affect seed bank dynamics.

Compositional trends

A three-dimensional NMDS ordination proved best for representing seed bank and vegetation species compositional variation. Stress of the final model was 13.4, corresponding to reasonably good preservation of between-sample relationships in ordination space (Clarke 1993). NMDS axes 1, 2, and 3 explained 36.1%, 16.9%, and 29.3% of variance, respectively. Accordingly, a combination of axis 1 with axis 3 provided the clearest two-dimensional representation for illustrating differences between trajectories for the seed bank and vegetation of different experimental treatments (Fig. 5).

For the sown treatments, but not for natural regeneration, seed bank and vegetation were distinctly and consistently different from each other in terms of species composition, as indicated by the fact that both were clearly separated in NMDS ordination space, with sown vegetation of all three sites consistently being located in the same part of ordination space (Fig. 5). A positive shift of vegetation trajectories along NMDS axis 1 was found for all treatments at the Norfolk Broads site, but only for the natural regeneration treatment at the other two sites (Fig. 5). As

indicated by the additional analyses of goodness-of-fit with the target community, this shift along the first NMDS axis coincided with an increase in compositional similarity with the respective NVC target communities (Fig. S1, Supporting Information).

For all three sites, trajectories differed strongly between natural regeneration and seeded treatments, and differed somewhat between ESA and NVC seeded treatments. Type of cultivation exerted little effect on these trajectories (Fig. 5).

Seed bank composition also shifted markedly towards higher NMDS axis 1 values (Fig. 5). For the Upper Thames Tributaries site, but not the other sites, seed bank composition also shifted towards higher values along NMDS axis 3 (Fig. 5).

The corresponding species plot (Fig. 6) summarizes the underlying patterns and trends at the species level. The positive shift in seed bank composition along NMDS axis 1 was partly affected by declining seed bank densities of unsown ruderal species positioned on the left-hand side, including *Capsella bursa-pastoris*, *Lamium purpureum*, *Poa annua*, and *Polygonum aviculare* (Fig. 6). At the same time, it was also partly affected by increasing seed bank densities of several sown target species located on the right-hand side of the plot (Fig. 6). Of these, *Agrostis capillaris*, *Cynosurus cristatu*s, *Festuca ovina/rubra* agg., *Hypochaeris radicata*, *Leucanthemum vulgare* and *Plantago lanceolata* all had accumulated substantial seed bank densities of up to several hundred seeds per m^2 by 1998, whereas several others, including *Anthoxanthum odoratum*, *Prunella vulgaris* and *Trifolium dubium* appeared to have accumulated more gradually (Table S6, Supporting Information).

Seed bank compositional shifts along NMDS axis 3 for the Upper Thames Tributaries site were driven by the rapid decline in seed bank densities of a number of ruderal species largely limited to this site and located in the lower half of the plot, including *Helminthotheca echioides*, *Juncus articulatus*, and *Persicaria maculosa* (Fig. 6). The

generally higher NMDS axis 3 scores for the vegetation than for the seed bank at the three sites were affected by several sown species located at the top of the plot, including *Alopecurus pratensis*, *Phleum pratense*, *Poa pratensis*, and *Schedonorus pratensis* – that had successfully established in the vegetation of one or more sites, but hardly accumulated any seeds in the soil (Table S6).

Discussion

Seed mixtures

The sowing of seed mixtures was associated with a decline of unsown species in the seed bank, both in terms of seed densities and average species richness per sample. Compared to natural regeneration, sowing of both the grass-only ESA mixtures and of the diverse grass-forb NVC mixtures led to a decline in seed densities of unsown grasses. At the same time, decline in unsown forb densities may have been somewhat slower with ESA mixtures than with NVC mixtures, but our findings are somewhat inconclusive, with analyses A and B yielding seemingly contradictory results.

Overall, our findings on the effects of seed mixtures on seed density dynamics of unsown species groupings are in agreement with other studies showing that the presence of particular functional groups of species may increase resistance to further colonization by other members of the same functional group, as e.g. shown for graminoids (Helsen et al. 2016) and for legumes (Turnbull et al. 2005). For our results, this means that lower seed bank densities of unsown forbs and grasses in seeded restoration treatments may have been the result of competitive exclusion which would have prevented the continued replenishment of existing seed reserves of unsown species, resulting in faster net depletion.

Interestingly, the seeds of sown forbs accumulated at a lower rate in the soil on treatment plots sown with grass-only ESA mixtures than on naturally regenerating plots. This may have been a consequence of negative priority effects exerted by sown grasses on sown-forb colonization from adjacent NVC plots. In another grassland restoration experiment on ex-arable land, carried out by Werner et al. (2016), grasses exerted very strong negative priority effects on forbs. In fact, negative priority effects from sowing grass-dominated seed mixtures can effectively prevent partially restored grassland from further progressing towards forb-rich stages that would more strongly resemble the target vegetation (Fagan et al 2008). Our own findings may reflect such processes, albeit at a smaller spatial and temporal scale.

A potentially higher species richness of sown grasses in seed bank samples from NVC-sown treatments, compared with those from ESA-sown treatments indicated by our results most likely simply represents an artefact due to NVC mixtures having included more grasses than ESA mixtures.

Cultivation

With the exception of a weak effect on seed densities of unsown grasses, depth of cultivation (deep ploughing versus shallow cultivation using discs or harrows) appeared to have little effect on seed bank densities of sown or unsown groups, nor did it affect compositional trajectories. This may be due to the fact that deep cultivation by ploughing was carried out to a depth of no more than 30-40 cm, and may thus have hardly extended below the regular depth of cultivation in the years prior to the experiment when sites were still managed as arable land. This means that the vertical distribution of seeds across the actively managed soil profile may have been fairly homogeneous at the start of the experiment, and therefore the two

cultivation treatments may have had little effect in terms of vertical re-distribution of seeds in the soil profile. Accordingly, Glen (2008) found that, compared to deep-ploughing to greater depths, conventional ploughing has very limited effects on the seed bank in ex-arable restoration.

Compositional trends

Species composition of the vegetation of sown treatments was rather static throughout the four years in which vegetation was monitored, with the exception of that at the Norfolk Broads site, where compositional similarity with the target community continued to increase. Nonetheless, in these sown treatments, any observed compositional convergence between the soil seed bank and the vegetation was rather limited. This is not surprising as compositional similarity between these two compartments usually remains quite low even in old grassland (Hopfensperger 2007; Kiss et al. 2017). This is due to i) the fact that only a limited number of specialist grassland species tend to form persistent seed banks (Bekker et al. 1998), and ii) the fact that the seeds of early-successional ruderal species often persist in the soil for decades after conversion from arable to grassland has taken place (Chancellor 1986). For the seed bank, compositional shifts during restoration involved both an increase in seed densities of sown species as well as a decline in seed densities of unsown species. Both trends were more pronounced in sown treatments than under natural regeneration, reflecting both a suppression of unsown species and a boost to seed bank formation of late-successional sown species from sowing. Without sowing, such late-successional target species tend to build up only slowly in the seed bank during restoration, reflecting the slowness of unassisted colonization (Helsen et al. 2015).

With respect to dynamics of different groups of unsown species relative to each other, unsown forbs tended to decline faster than unsown grasses. This may have been due to unsown forbs in our study having mainly been ruderal species confined to the seed bank, whereas unsown grasses also contained generalist grassland species such as *Holcus lanatus* that established rather well in the vegetation, enabling them to replenish their buried seed reserves. Observed declines in seed bank densities of ruderal species were broadly in line with declines observed by other authors (Akinola et al. 1998). From the limited information available on seed bank composition of mature MG5 grassland (Kirkham & Kent 1997), it appears that with the exception of rushes (*Juncus* spp.), ruderal species tend to be poorly represented in the seed bank of MG5 reference sites. On the other hand, a number of component species of MG5 communities species which were sown in our study, such as *Agrostis capillaris, Cynosurus cristatus* and *Plantago lanceolata,* and which readily accumulated seeds in the soil once successfully established, are also prevalent in the seed bank of mature MG5 grassland (Kirkham & Kent 1997). This suggests that over the seven years covered by our study, the seed bank at our experimental sites, particularly on plots seeded with the NVC mixtures, has made some compositional progress towards the desired reference condition.

Seed bank monitoring in the post-restoration phase

As suggested by Rayburn et al. (2016), seed bank analysis can complement more traditional methods of vegetation monitoring. Vegetation monitoring tends to focus on species presence in the vegetation, but usually fails to take into account seed production and recruitment aspects, which are important when assessing restoration progress under functional aspects (Godefroid et al. 2011). Time required by sown

547 target species to become self-sustaining, i.e. capable of regeneration e.g. by self-
548 seeding does not just depend on initial establishment and survival, but also on how
549 well species requirements are met during restoration (Wagner et al. 2016). Seed bank
550 monitoring, by indicating whether species have succeeded in building up a functional
551 soil seed bank, can complement the information provided by traditional vegetation
552 monitoring, thus allowing for a more integrated assessment of restoration progress
553 also in functional terms. It can help differentiate between those populations that are
554 fully functionally restored and those that may only just manage to persist under sub-
555 optimal conditions. While inclusion of seed bank monitoring does involve additional
556 time and resources, it is worth doing so where such resources are available.
557

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563

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692 **Supporting Information**

693 The following information may be found in the online version of this article:

694

695 Figure S1. Goodness-of-fit to NVC target communities for the vegetation in the
696 various restoration treatments at each site.

697 Table S1. Composition of species-poor ESA mixtures and of the species-rich NVC
698 mixtures used in sown treatments at the three experimental sites.

699 Table S2. Results of mixed model analyses A of the effects of seed addition on seed
700 bank density and species richness of unsown species groupings.

701 Table S3. Results of mixed model analyses B of the effects of seed mixture type and
702 cultivation depth on seed bank density and species richness of unsown species
703 groupings.

704 Table S4. Results of mixed model analyses A of the effects of seed addition on seed
705 bank density and species richness of sown species groupings.

706 Table S5. Results of mixed model analyses B of the effects of seed mixture type and
707 cultivation depth on seed bank density and species richness of sown species groups.

708 Table S6. Seed bank densities and quadrat cell counts in the vegetation of all taxa
709 recorded in the experiment.

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Figure 1. Buried seed densities in the various restoration treatments for (a) all unsown species, (b) unsown forbs, and (c) unsown grasses. Error bars indicate ±SE (n = 12). Seed densities (a) also include *Juncus* spp. and woody species.

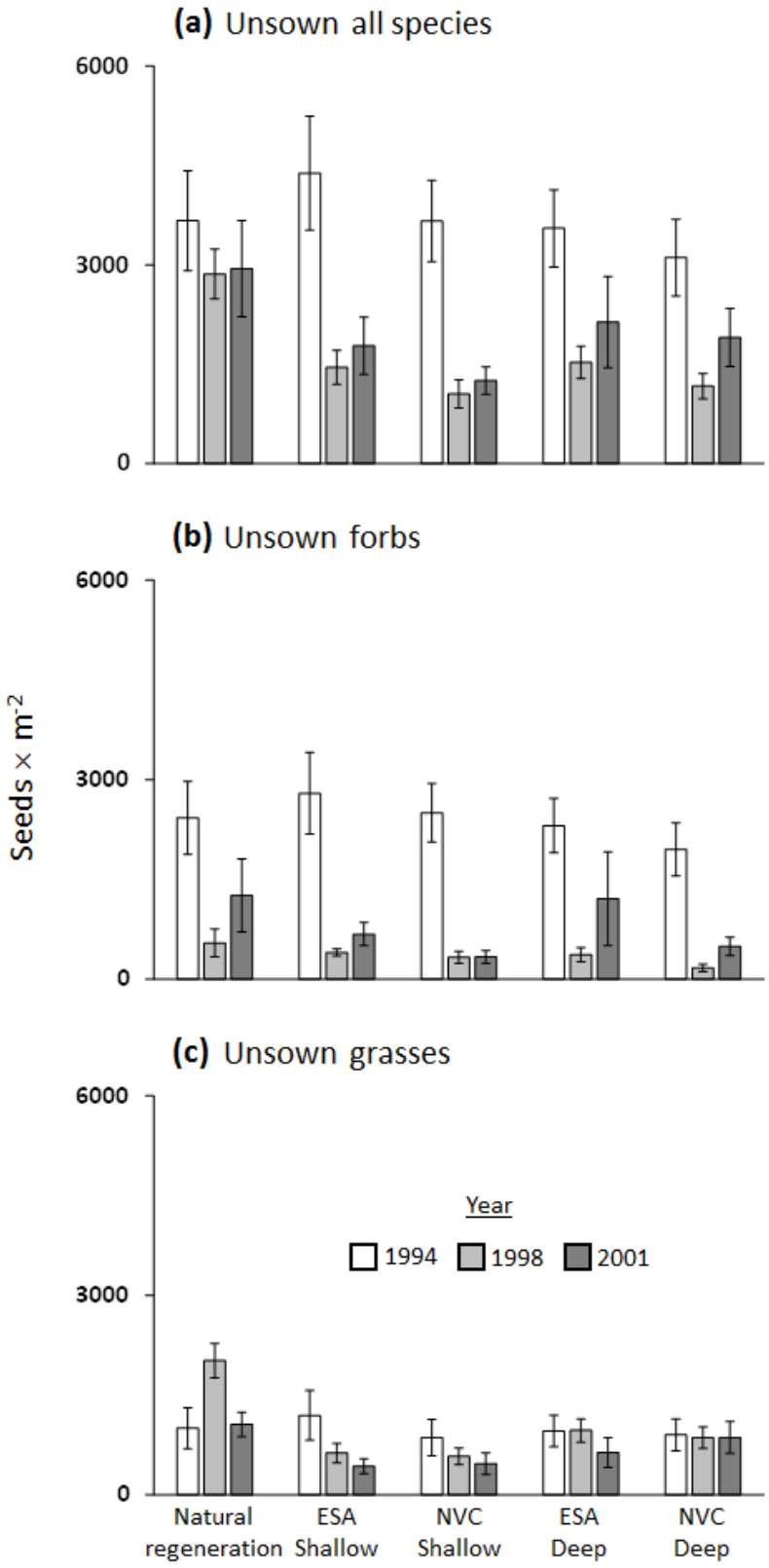

Figure 2. Sample species richness in the various restoration treatments for (a) all unsown species, (b) unsown forbs, and (c) unsown grasses. Error bars indicate ±SE (n = 12). Species counts for (a) also include *Juncus* spp. and woody species.

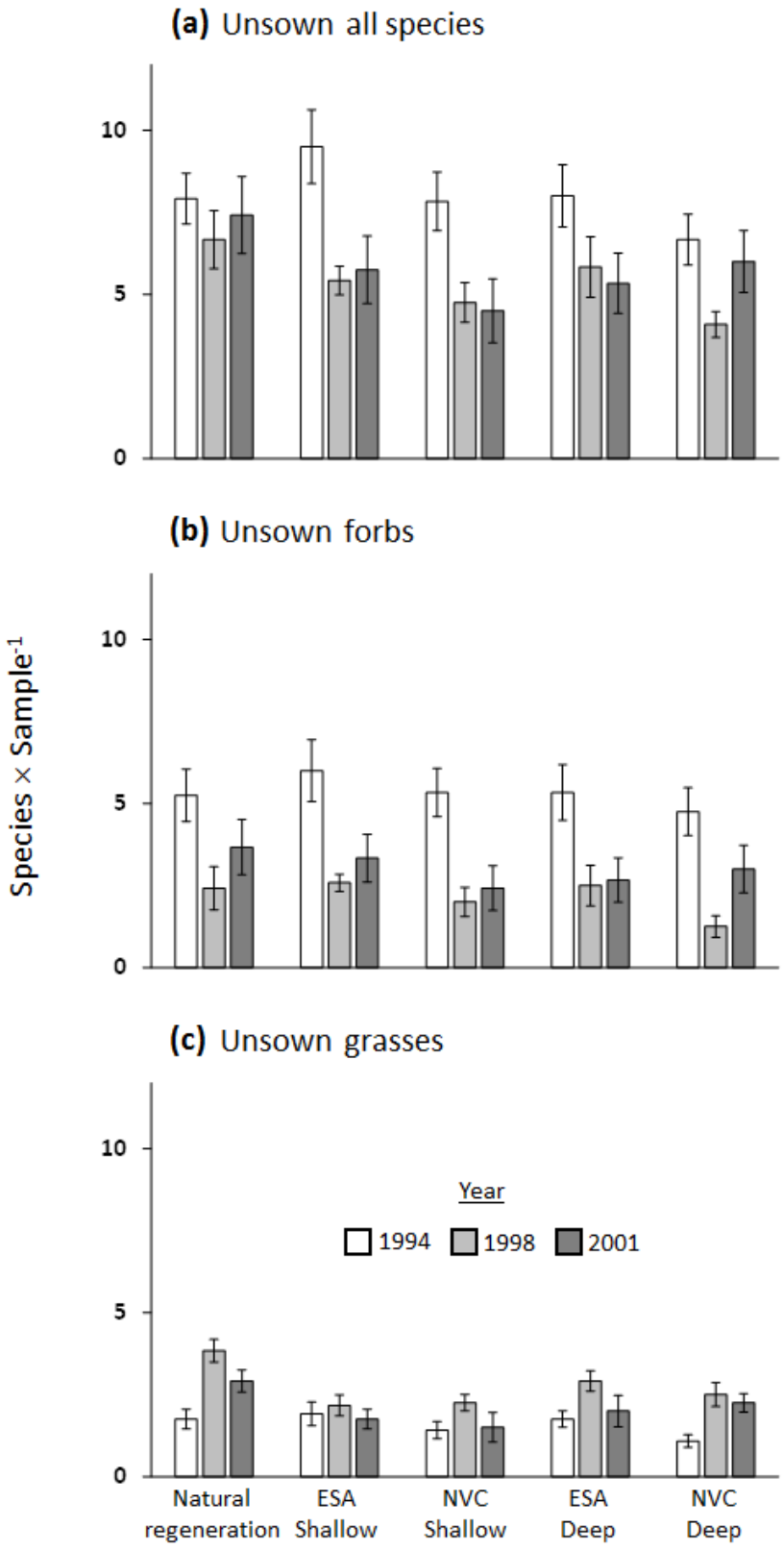

Figure 3. Buried seed densities in the various restoration treatments for (a) all sown species, (b) sown forbs, and (c) sown grasses. Error bars indicate ±SE (n = 12).

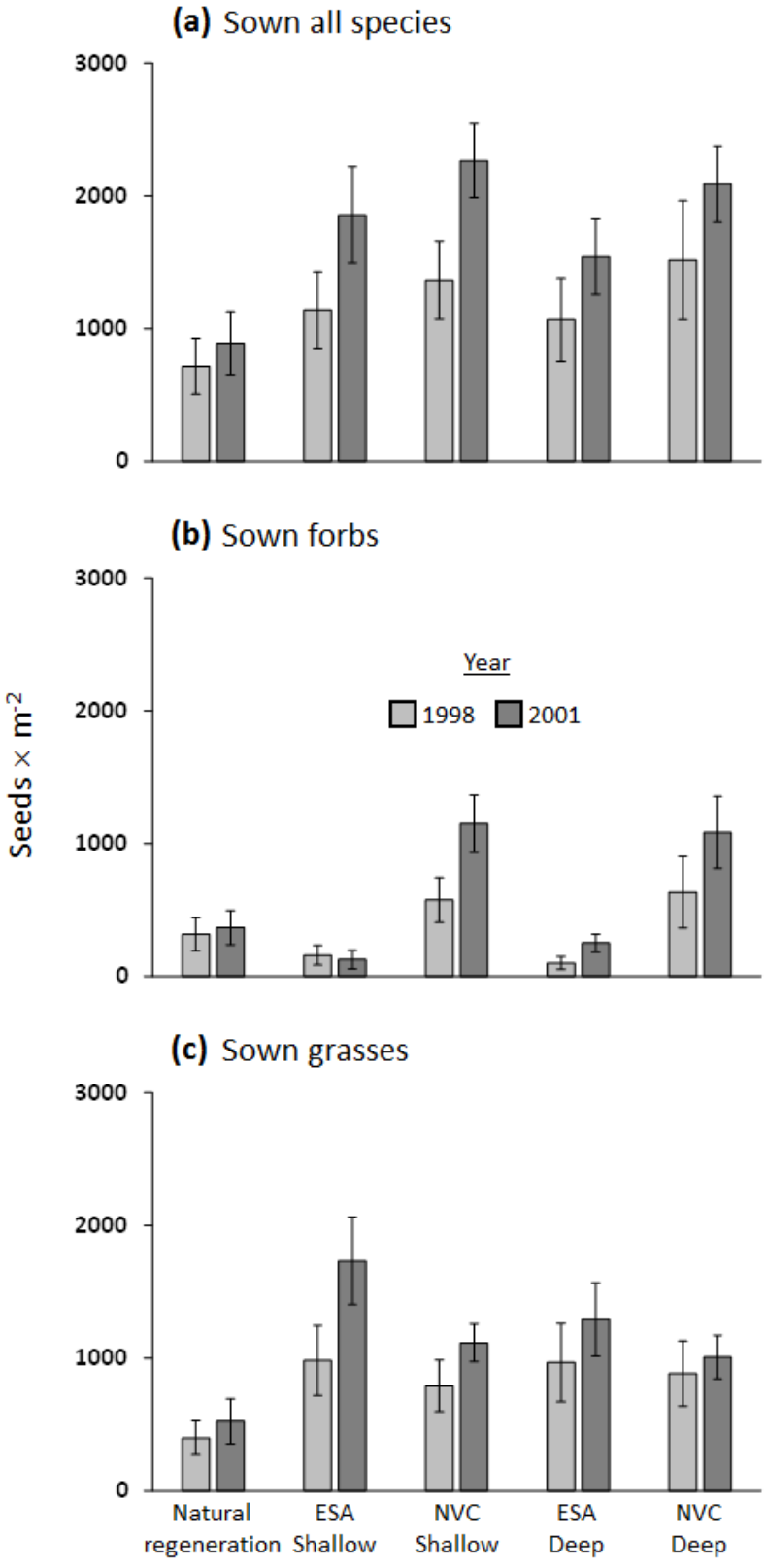

Figure 4. Sample species richness in the various restoration treatments for (a) all sown species, (b) sown forbs, and (c) sown grasses. Error bars indicate ±SE (n = 12).

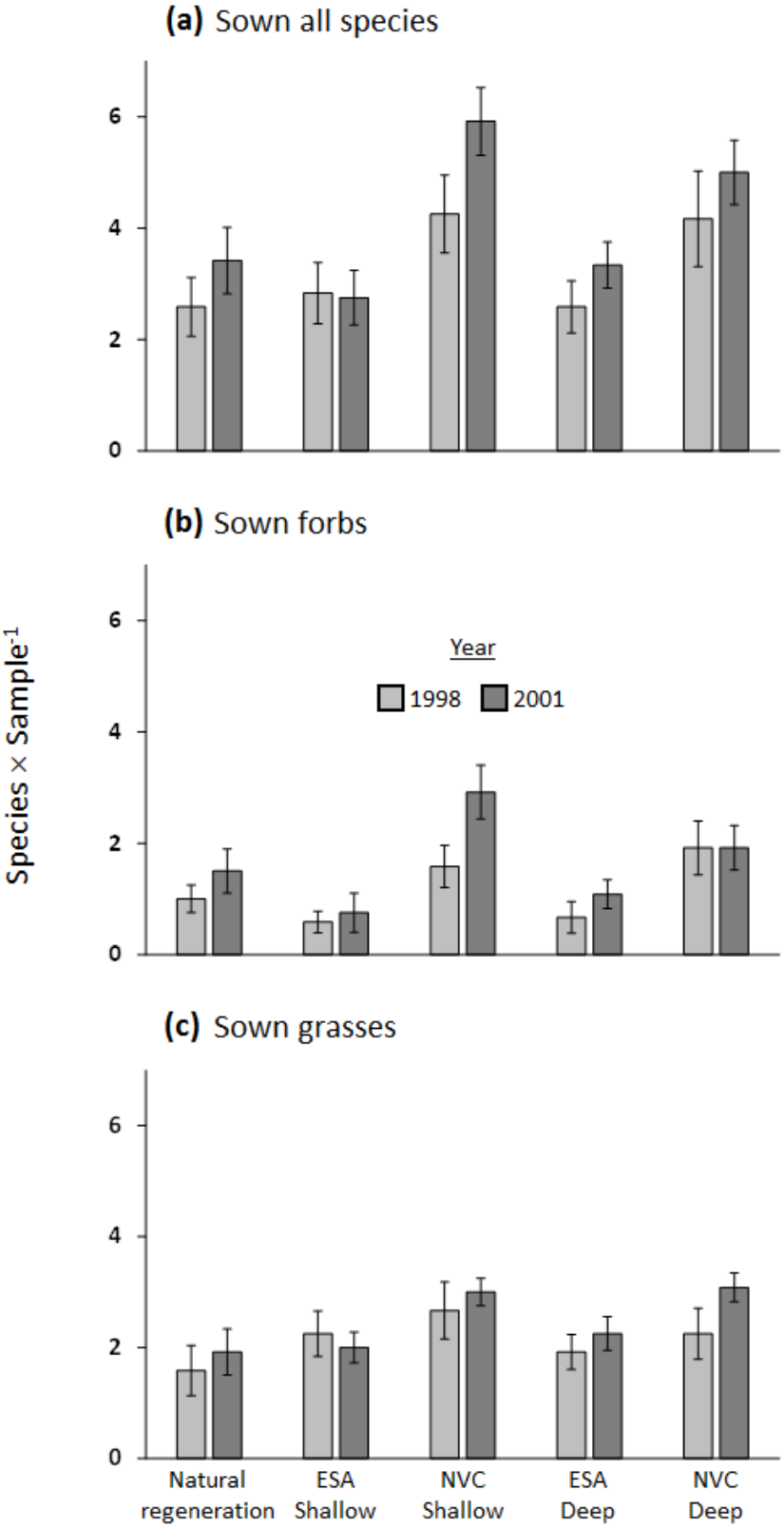

Figure 5. NMDS sample plots of a joint analysis of seed bank and vegetation at the three experimental sites. For clarity, trajectories are depicted separately for each site in panels (a) to (c). Axes 1 and 3 explain 36.1% and 29.3% of species composition, respectively.

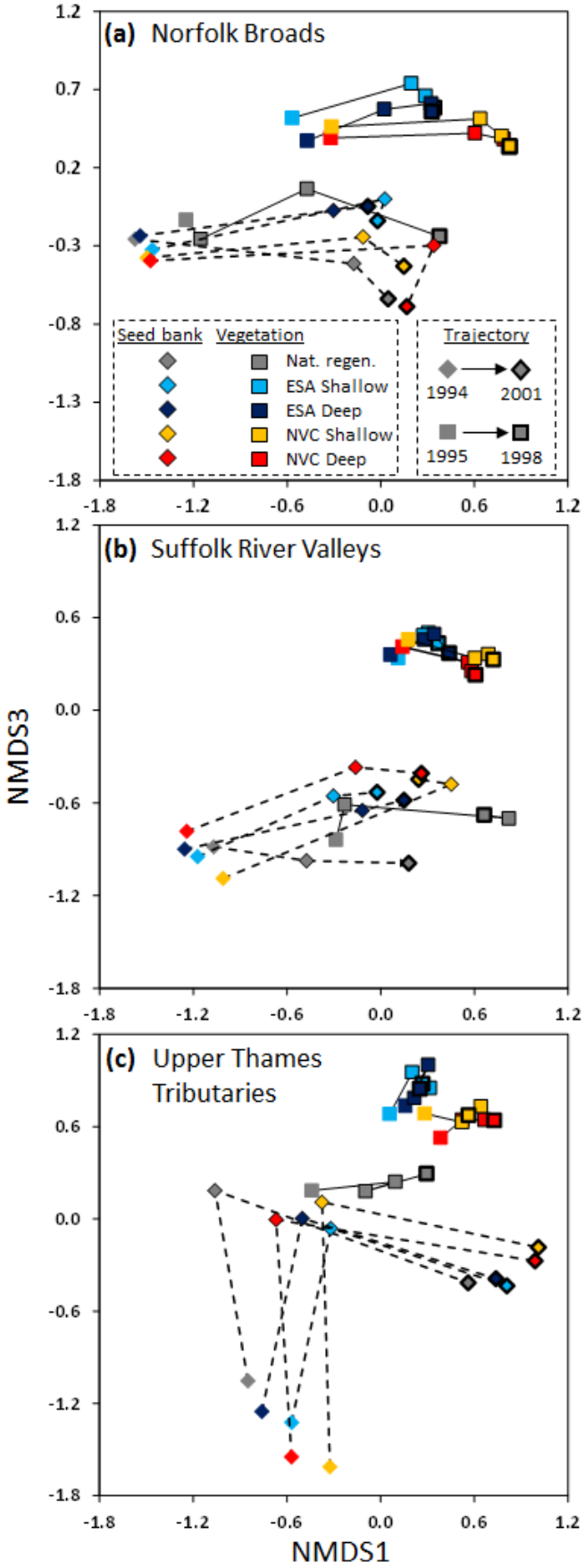

Figure 6. NMDS species plots of a joint analysis of seed bank and vegetation at the three experimental sites. To avoid overcrowding in this plot, only those species are plotted that did occur in at least ten vegetation and/or seed bank samples. Letter codes indicate species identity (for full names see Table S6, Supporting Information).

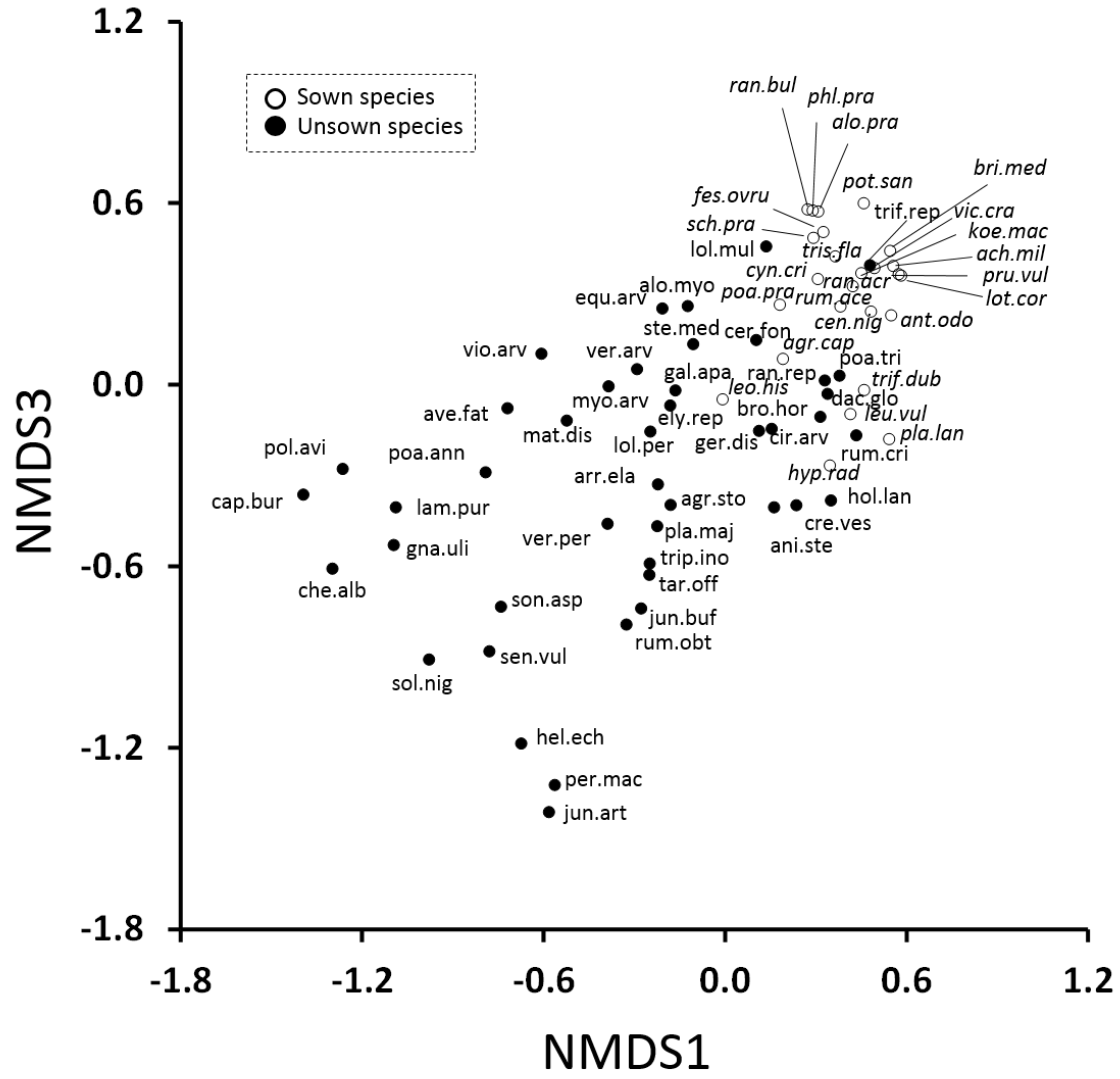

Table 1. Description of the three experimental sites.

Site	Location	Elevation (m a.s.l.)	Annual rain (mm)	Soil type	NVC target community (Rodwell 1992)
Norfolk Broads	52°44'N 1°36'E	< 5	500-600	Humic alluvial gley	MG5a *Cynosurus cristatus–Centaurea nigra* grassland: *Lathyrus pratensis* subcommunity
Suffolk River Valleys	52°01'N 1°20'E	<10	500-600	Brown sand	MG5c *Cynosurus cristatus–Centaurea nigra* grassland: *Danthonia decumbens* subcommunity
Upper Thames Tributaries	51°52'N 1°03'W	70	600-700	Alluvial gley	MG5a *Cynosurus cristatus–Centaurea nigra* grassland: *Lathyrus pratensis* subcommunity